EXHIBIT A156

TOXICOLOGY AND APPLIED PHARMACOLOGY 147, 143–150 (1997)
ARTICLE NO. TO978276

Mineralogical Features Associated with Cytotoxic and Proliferative Effects of Fibrous Talc and Asbestos on Rodent Tracheal Epithelial and Pleural Mesothelial Cells

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Received May 12, 1997; accepted August 11, 1997

Mineralogical Features Associated with Cytotoxic and Proliferative Effects of Fibrous Talc and Asbestos on Rodent Tracheal Epithelial and Pleural Mesothelial Cells. Wylie, A. G., Skinner, H. C. W., Marsh, J., Snyder, H., Garzione, C., Hodkinson, D., Winters, R. and Mossman, B. T. (1997). *Toxicol. Appl. Pharmacol.* 147, 143–150.

Inhalation of asbestos fibers causes cell damage and increases in cell proliferation in various cell types of the lung and pleura *in vivo*. By using a colony-forming efficiency (CFE) assay, the cytotoxicity and proliferative potential of three mineral samples containing various proportions of fibrous talc were compared to NIEHS samples of crocidolite and chrysotile asbestos in cell types giving rise to tracheobronchial carcinomas, i.e., hamster tracheal epithelial (HTE) cells, and mesotheliomas, i.e., rat pleural mesothelial (RPM) cells. Characterization of mineralogical composition, surface area, and size distributions as well as proportions of fibers in all mineral samples allowed examination of data by various dose parameters including equal weight concentrations, numbers of fibers >5 μm in length, and equivalent surface areas. Exposure to samples of asbestos caused increased numbers of colonies of HTE cells, an indication of proliferative potential, but fibrous talc did not. RPMs did not exhibit increased CFE in response to either asbestos or talc samples. Decreased numbers of colonies, an indication of cytotoxicity, were observed in both cell types and were more striking at lower weight concentrations of asbestos in comparison to talc samples. However, all samples of fibrous minerals produced comparable dose–response effects when dose was measured as numbers of fibers greater than 5 μm or surface area. The unique proliferative response of HTE cells to asbestos could not be explained by differences in fiber dimensions or surface areas, indicating an important role of mineralogical composition rather than size of fibers. © 1997 Academic Press

Occupational exposures to mineral fibers such as asbestos are associated with the development of pulmonary and pleural disease (Mossman and Gee, 1989; Mossman *et al.,* 1990; Guthrie and Mossman, 1993). Although various types of asbestos are biologically active in a number of *in vivo* and *in vitro* bioassays, the properties of fibers important in reactivity with cells and tissues are unclear (Guthrie and Mossman, 1993; Mossman and Begin, 1989). It is generally agreed that length and width or aspect ratio are important variables for predicting the carcinogenicity and fibrogenicity of durable fibers (Davis *et al.,* 1986; Stanton *et al.,* 1981). However, the mineralogical composition and structural features of fibers and particles may also play a role in pathogenicity (Oehlert, 1991; Wylie *et al.,* 1987; Skinner *et al.,* 1988; Wylie *et al.,* 1993). These properties govern surface properties as well as durability of fibers in the lungs and pleura, factors that may be critical in the development of lung cancer and mesothelioma. (Mossman and Gee, 1989; Mossman *et al.,* 1990; Guthrie and Mossman, 1993; Health Effects Institute, 1991).

Asbestos types, in contrast to a number of other fibrous and nonfibrous nonpathogenic materials, cause both cell proliferation and cytotoxicity in a dose-related fashion in several cell types (reviewed in Health Effects Institute, 1991). These biological responses may reflect the disease potential of various fiber types, as cell injury and hyperplasia are early events in rodent inhalation models of asbestosis and carcinogenesis (Mossman and Gee, 1989; Mossman *et al.,* 1990; Guthrie and Mossman, 1993; Health Effects Institute, 1991). In this study, we compared the cytotoxicity and proliferative potential of three New York talc samples to crocidolite and chrysotile asbestos in cell types affected in asbestos-induced tumors, i.e., hamster tracheal epithelial (HTE) cells, which can give rise to tracheobronchial neoplasms, and rat pleural mesothelial (RPM) cells, cells affected in the development of mesothelioma. In studies here, we used an established colony-forming efficiency (CFE) assay that documents both increases in cell proliferation and cell survival, as measured by increases in numbers of colonies, at low concentrations of minerals, and growth inhibition, as indicated by decreases in colony formation or size at high concentration of minerals, to compare responses to well-characterized samples of asbestos and fibrous talc in HTE and RPM cells. An additional advantage of this bioassay is that it employs cells from the lung and pleura and measures responses

0041-008X/97 $25.00

to minerals over a 7-day time period of exposure as opposed to shorter time frames used (<24 hr) in most other *in vitro* assays in the literature (reviewed in Health Effects Institute, 1991. In the CFE assay, nonfibrous particles such as glass beads are proliferative or cytotoxic to HTE cells at ⩾100-fold concentrations when compared to asbestos at equal weight concentrations (Mossman and Sesko, 1990; Marsh *et al.,* 1994; Timblin *et al.,* 1995).

The three talc samples used here differ somewhat in their mineralogy, both in the types of minerals and in their relative abundances. However, all three contain varying proportions of fibrous talc which is similar dimensionally and morphologically to asbestos. We thus hypothesized that factors other than length and width of fibers would govern the reactivity of minerals in the *in vitro* assays used here. The experiments were undertaken to explore the questions: (1) Do fibrous talc and asbestos fibers cause similar biological responses in epithelial and mesothelial cells? (2) Is reactivity to mineral samples dose related? and (3) Are responses in various cell types related only to numbers and sizes of fibers in each preparation or does mineralogy, including chemical composition, surface properties, and mineral structure, play a role?

METHODS

Sources of Mineral Samples

Three samples from the New York State Gouverneur Mining District, FD14, S157, and CPS183, and two asbestos samples, NIEHS chrysotile (Plastibest 20) and NIEHS crocidolite, were used in this study. The asbestos samples are essentially monomineralic and have been studied in detail (Campbell *et al.,* 1980). The general geology and mineralogy of the Gouverneur District are described by Engle (1962) and Ross *et al.* (1968). FD14 is a commercial talc, S157 was once produced from this district as a fiber talc product, and CPS183 is a laboratory separated concentrate of fibrous talc. Fibrous talc is a general term that includes fibers composed entirely of the mineral talc as well as fibers that are composed of both talc and amphibole (probably anthophyllite) intergrown on a submicrometer scale (Stemple and Brindley, 1960; Virta, 1985). The index of refraction of the fibers increases as the amphibole component increases (Veblen and Wylie, 1993). Fibrous talc is present in trace amounts in many commercial talc deposits, but it is a major component of most talc products from the Gouverneur Talc District. All samples were characterized by scanning electron microscopy (SEM), optical microscopy (OM), and x-ray diffraction (XRD); CPS183 and NIEHS crocidolite were also studied by TEM as this technique is more sensitive for the detection of smaller, thinner particles.

Characterization of Minerals

The samples were studied by XRD and SEM at Yale University in order to establish the overall mineralogy, mineral abundances, and the number of fibers per microgram. They were examined by OM at the Laboratory for Mineral Deposits Research, University of Maryland, in order to determine the mineralogy, mineral abundances, and number of fibers per microgram of the samples, and by transmission electron microscopy (TEM) at AMA Laboratories, Beltsville, Maryland (under the direction of the Laboratory for Mineral Deposits Research) for the purpose of determining the detailed size distribution of fibrous talc and especially to examine the content of fibers 0.1 μm in width and smaller. The protocols followed in each laboratory are described below. For purposes of this paper, ''particles'' refers to particles of all aspect ratios. ''Fiber'' refers to particles that have an aspect ratio (length/width) of at least five and to bundles of such fibers. ''Fibers'' (unless otherwise specified) include true mineral fibers (very high aspect ratio particles whose shapes were attained during mineral formation) as well as elongated cleavage fragments (shape produced during comminution).

X-ray diffraction. Samples mixed with an internal standard and spun to minimize preferred orientation were analyzed by using a SCITAG Pad V automated diffractometer. Identification of minerals was based on comparison of the X-ray pattern with standard patterns.

Optical microscopy. A known weight of sample was dispersed in water and then passed through a 22-gauge needle 8× and sonicated 4 min before mounting on slides. A drop of immersion oil $n_D = 1.598$ was placed over the dried sample. For all samples except chrysotile ($N = 2$ mixtures), at least five separate mixtures were prepared from each sample and at least two slides were made from each mixture. One-hundred fibers were counted from each slide. All fibers longer than 5 μm and all particles that appeared to be composed of bundles of fibers were categorized by length and width and by index of refraction according to the following characteristics: all indices of refraction greater than 1.598 (amphibole), index of refraction parallel to elongation greater than 1.598 and index of refraction perpendicular to elongation less than 1.598 (fibers composed of talc and a significant amount of amphibole, and referred to as talc/amphibole), or all indices of refraction less than 1.598 (fibers dominated by the mineral talc). The number of fiber per microgram was calculated by assuming that particle distributions were representative and directly proportional to the area of the filter.

Scanning electron microscopy. A known weight of sample was dispersed in water, passed through a 22-gauge syringe needle 8×, and deposited onto a 0.45-μm cellulose filter. Replicate preparations were made for each sample and analyzed independently to test for homogeneity. The filters were examined with a JEOL JXA 8600 SEM equipped with EDXA. Particles that were at least 1 μm in length and 0.12 μm in width could be detected. Mineral identification was automated by predetermining the relative percentages of Na, Ca, K, Mg, Al, Si, Mn, and Fe in mineral standards and comparing them to the elemental compositions determined on the sample particles (Petruk and Skinner, 1997). The number of particles per microgram of sample was calculated by assuming that the particle distributions were representative and directly proportional to the area of the filter.

Transmission electron microscopy. A known weight of sample was dispersed in water, flushed with a 22-gauge syringe needle 8×, and then sonicated for 4 min. The solutions were then diluted and filtered through a 0.22-μm cellulose acetate filter. The samples were analyzed on a JEOL 100 CX II electron microscope at 19,000× magnification. Over 300 fibers from each sample were measured.

Surface area measurements. All five samples were tested for single point N_2-BET surface areas by J. W. Anderson of R. T. Vanderbilt Corporation. The tests were repeated 4× for each sample. Data were expressed as square millimeters per gram of sample.

Cell culture and addition of fibers to bioassay. A HTE cell line previously isolated and characterized by Mossman *et al.* (1980) was maintained at passages from 38 to 50 and cultured routinely in Ham's F12 medium (Gibco, Grand Island, NY) containing penicillin and streptomycin (both at 100 U/ml) and 10% newborn calf serum (Gibco). This cell line is diploid and possesses features, i.e., mucin secretion and cilia, of differentiated epithelial cells. Primary cultures of RPM cells were isolated by scraping the parietal pleural of two weanling male Fischer 344 rats (Janssen *et al.,* 1994) and were maintained for up to eight passages in Ham's F12–DMEM containing antibiotics (as above), 10% fetal calf serum (Gibco), hydrocortisone (100 ng/ml), insulin (2.5 μg/ml), transferrin (2.5 μg/ml), and selenium (2.5 ng/ml).

Mineral samples presterilized in a dry oven overnight at 130°C were added to Hanks' balanced salt solution (HBSS) before titration 8× through a 22-gauge syringe needle and addition to cultures in 2% serum-containing medium.

A CFE assay was also used as a sensitive test for cytotoxicity and cell proliferation (Mossman and Sesko, 1990; Marsh *et al.,* 1994; Timblin *et al.,* 1995). HTE (400 cells/60 mm dish) and RPM (2000/60 mm dish) were plated for 24 hr before addition of dusts to medium containing 2% serum as described

TABLE 1
Characterization of Talc and Asbestos Samples

Sample	Mineralogy
	Mineral composition (% of sample)
FD14	Talc (37), tremolite (35), serpentine (15), other (<2), unknown (12)[a]
S157	Talc (60), tremolite (12), unknown (21), other (4), anthophyllite (3), quartz (1)
CPS183	Talc (50), quartz (12), unknown (28), tremolite (4), other (4), anthophyllite (3)
NIEHS crocidolite	Riebeckite (100)
NIEHS chrysotile	Chrysotile (100)
	Mineralogy of fibers >5 μm (% of fibers)
FD14	Talc (62), amphibole (24),[b] talc/amphibole (14)
S157	Talc (84), amphibole (11), talc/amphibole (5)
CPS183	Talc (99), amphibole (1), talc/amphibole (<1)
NIEHS crocidolite	Crocidolite (100)
NIEHS chrysotile	Chrysotile (100)

Sample	Surface area (mm^2/g)	Fibers/μg[c]	Fibers ≥ 5 μm/μg
FD14	6.2 ± 0.2[d]	2.5×10^3	0.8×10^3
S157	4.9 ± 0.2	1.1×10^4	4.8×10^3
CPS183	4.9 ± 0.4	1.1×10^4	9.2×10^3
NIEHS crocidolite	10.3 ± 1.3	5.3×10^5	3.8×10^5
NIEHS chrysotile	25.4 ± 0.5	5.3×10^4	3.4×10^4

[a] Primarily magnesium silicates (talc and talc/amphibole) with SEM/EDXA spectra too low for conclusive identification.

[b] The most abundant amphibole is tremolite. A very small amount of anthophyllite may be included.

[c] Data are based on SEM measurements. Chrysotile values are low due to its poor visibility on the SEM. Standard error of measurement is estimated to be 20%.

[d] Mean ± standard error of measurement of four individual measurements per group.

above. Minerals were then added, and untreated and mineral-exposed cultures were maintained for 7 days before examination. At this time, plates were rinsed in HBSS and fixed in methanol and stained with 10% Giemsa stain, and total colonies greater than 50 cells per plate were counted by using a blind code (Mossman and Sesko, 1990; Marsh *et al.,* 1994; Timblin *et al.,* 1995). Duplicate experiments were performed for each bioassay with $N = 3–4$ dishes per group per experiment. Statistical analyses of all data were performed by using analysis of variance and trend analysis.

RESULTS

Mineralogy

The overall mineralogical composition, the mineral composition of the fibers, the number of fibers per microgram, and the surface area measurement of the samples used in our studies are given in Table 1. FD14 is composed of platy talc, true mineral fibers of talc and talc/amphibole, cleavage fragments of tremolite, platy serpentine (chrysotile absent), and trace amounts of other minerals. Fibers make up approximately 11% of the particles identified by SEM. They are mostly talc followed by amphibole cleavage fragments and talc/amphibole. S157 is composed of platy talc, true mineral fibers of talc and talc/amphibole, tremolite and anthophyllite cleavage fragments, and quartz. Fibers make up about 37% of the particles, and they are mostly talc with smaller amounts of amphibole cleavage fragments and talc/amphibole. CPS183 is composed of true mineral fibers of talc and a very small amount of talc/amphibole, cleavage fragments of tremolite and anthophyllite, and quartz. Fifty-nine percent of the particles are fibers, and they are almost all fibers of talc. The three talc samples represent a range in the amount of fiber present (both in portion of sample and in number of fibers/μg) and in the mineralogy of the fibrous portion, primarily in the content of amphibole both as a separate phase and as a component of fibrous talc. NIEHS crocidolite and NIEHS chrysotile are essentially monomineralic populations of true mineral fibers of riebeckite and chrysotile, respectively. The very small widths result in many more fibers per microgram than are found in the talc samples.

Surface Area

The specific surface areas (mm^2/g) of talc samples are smaller than asbestos samples and roughly comparable to each other. The larger surface area of FD14 compared to the other talc samples is probably due to the presence of more abundant small platy talc particles that have two almost equivalent dimensions and one that is very much smaller, producing a large surface area/mass ratio. The greater surface area of chrysotile with respect to crocidolite can be attributed to its lower density and small fibril width and perhaps in part to the straw-like structure of the chrysotile fibers if N_2 penetrates the hollow center of the chrysotile tubes. Since the surface reactivity of different minerals affects the surface adsorption of N_2, some of the variation among samples may be related to mineralogy as well.

Size Distributions of Fibers in Mineral Preparations

Figure 1 shows the frequency of length and width for all fibers in units of fibers/microgram and the frequency of width for only those fibers greater than or equal to 5 μm in length as established by SEM and OM. The abundance of narrow crocidolite fibers accounts for the fact that the NIEHS crocidolite contains more fibers per microgram than any other sample (Table 1). CPS183 and S157 are very similar in many respects. They are composed of similar numbers of fibers per microgram, but there are slightly more longer fibers and fewer long, wide fibers in CPS183. FD14 contains the smallest number of fibers per microgram and the highest proportion of the widest fibers. In general, talc fibers are narrower than amphibole cleavage fragments and the differences in the sizes of the fibers among the talc samples in part reflect the differences in the abundance of amphibole cleavage fragments vs fibrous talc. As the amphibole content increases from CPS183 to S157 to

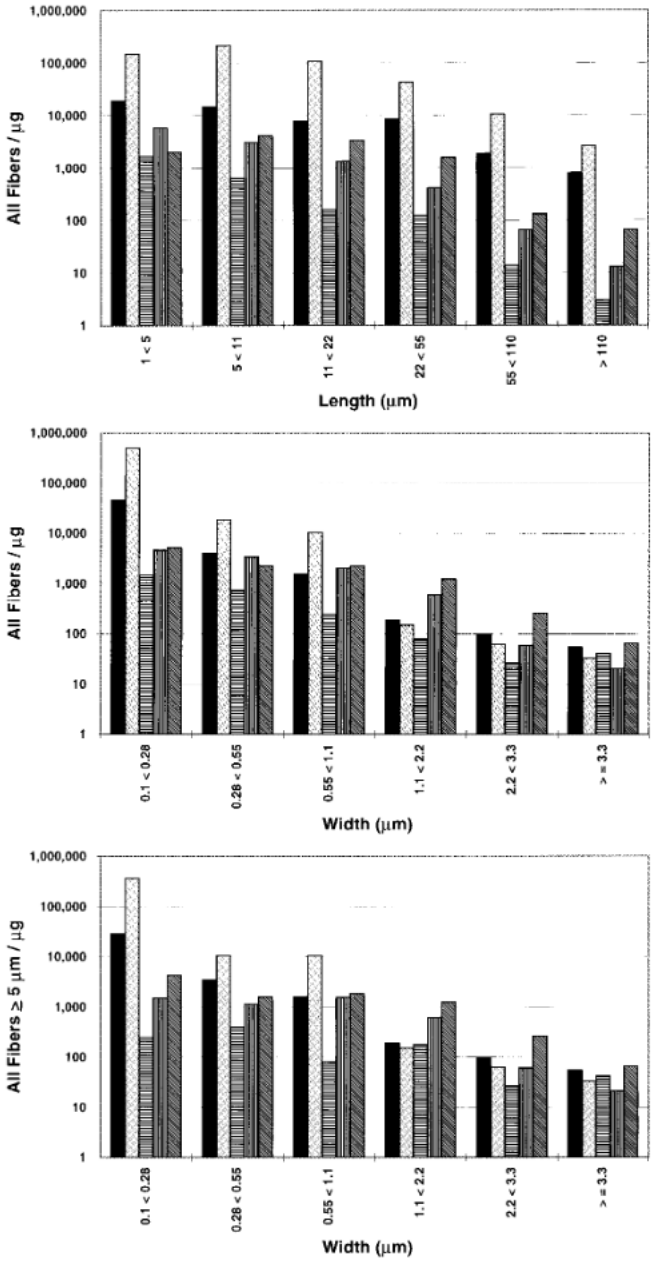

FIG. 1. The frequencies of length and width in units of fibers per microgram are shown for the three talc samples and two NIEHS asbestos samples. Also shown is the frequency of width (fibers/µg) for those fibers longer than 5 µm. (■) Chrysotile; (▤) crocidolite; (▤) FD14; (▥) S157; (▨) CPS183.

FD14, the total fiber content goes down, and, on average, the fibers decrease in length and increase in width. No distinctions between the size distributions of talc and talc/amphibole fibers were documented.

Table 2 gives the percentage of fibers in length–width categories for CPS183 and NIEHS crocidolite asbestos as measured by TEM. These data enable a direct comparison between the dimensions of fibrous talc and crocidolite that is not restricted by the 0.1-µm width limit in the SEM data. These two true mineral fiber populations are quite similar, differing most notably in the higher proportion of wide (>0.5 µm) fibers and slightly lower proportion of long (>20 µm) fibers in fibrous talc.

CFE Assays

Combined data from duplicate experiments with HTE and RPM cells are presented in Figs. 2 and 3, respectively. CFE data are expressed as a ratio of the number of colonies in mineral-exposed cultures in comparison to control colonies × 100 at various concentrations of minerals on a weight basis (µg/cm^2) as is typically found in the literature (Mossman *et al.,* 1990; Health Effects Institute, 1991). In HTE cells, both asbestos types showed an elevated number of colonies ($p < 0.05$) at lowest concentrations indicating increased cell proliferation and/or survival in response to asbestos fibers and confirming earlier studies (Mossman and Sesko, 1990; Marsh *et al.,* 1994). Significant decreases ($P < 0.05$) in CFE, an indication of toxicity or growth inhibition, were observed at concentrations of asbestos of 0.5 µg/cm^2 and greater. In contrast, RPM cells did not exhibit proliferative effects in response to either asbestos type, but statistically significant ($p < 0.05$) decreases in CFE were observed at concentrations of asbestos fibers greater than 0.05 µg/cm^2. In both cell types, the talc samples were less cytotoxic than asbestos. CPS183 was the most toxic talc sample, followed by S157 and FD14. In contrast to the other mineral samples, S157 and FD14 did not exhibit significant linear trends in cytotoxicity with increasing dosages in HTE cells.

Figures 4 and 5 show the same cellular response data as Figs. 2 and 3, but dose is calculated based on the number of fibers greater than or equal to 5 µm/cm^2 (fibers/cm^2) rather than total sample weight per square centimeter. The data are taken from the SEM characterizations, but the comparisons would be the same if OM or TEM data were used. Doses of total sample per square centimeter administered to the cultures

TABLE 2
Percentage of Fibers by Length and Width (µm) as Determined by Transmission Electron Microscopy

Length	Width: 0.01–0.1	>0.1–0.25	>0.25–0.5	>0.5–1.0	>1.0
CPS183					
<1	2.9	1.6	—	—	—
>1–2	4.1	14.1	0.5	—	—
>2–5	2.5	22.0	6.8	1.6	—
>5–10	0.9	9.8	4.3	4.5	0.5
>10–20	0.5	7.3	3.2	2.3	2.5
>20–50	0.2	1.8	2.7	1.4	2.0
>50–100	—	—	—	—	0.2
		NIEHS crocidolite			
<1	0.3	0.3	—	—	—
>1–2	1.1	9.5	0.3	—	—
>2–5	4.6	31.6	2.9	—	—
>5–10	1.4	18.1	3.7	0.6	—
>10–20	1.7	10.7	3.2	0.3	—
>20–50	0.6	2.9	1.4	1.1	—
>50–100	—	1.7	1.4	0.6	—

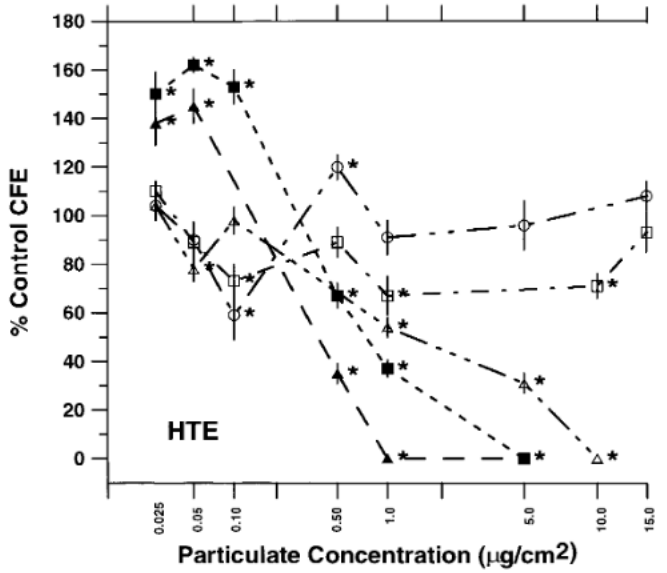

FIG. 2. Colony-forming efficiency (CFE) of HTE cells at various weight concentrations of samples. Standard error in CFE is indicated on symbol. *$p <$ 0.05 in comparison to untreated controls. (▲) Chrysotile; (■) crocidolite; (○) FD14; (□) S157; (△) CPS183.

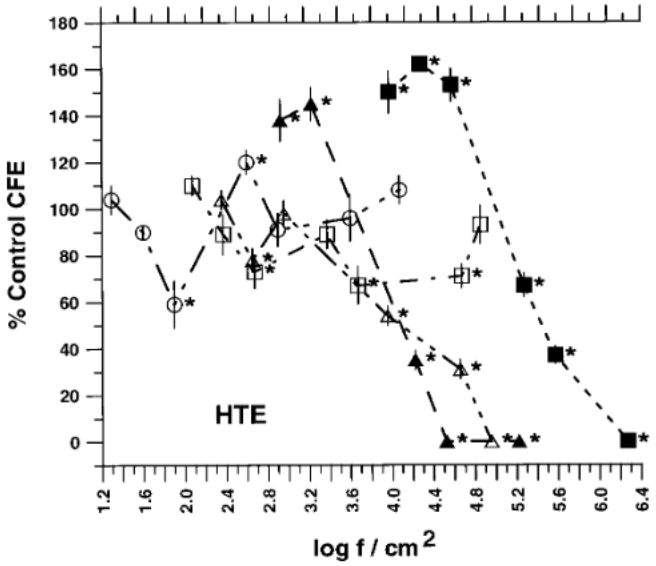

FIG. 4. Colony-forming efficiency (CFE) assays in HTE cells expressed as a function of fibers ≥5 μm in length per cm^2 (f/cm^2). The symbol width is equal to or greater than estimated error in f/cm^2. The standard error in CFE is indicated on the symbols. *$p <$ 0.05 in comparison to untreated controls. (▲) Chrysotile; (■) crocidolite; (○) FD14; (□) S157; (△) CPS183.

covered such a wide range that there were equivalent doses of fibers per square centimeter in almost all length/width categories for all samples. Therefore, even though crocidolite and chrysotile contained many more fibers per microgram than the talc samples, the same number of fibers per centimeter were administered in low doses of asbestos and high doses of talc (μg/cm^2).

As shown in Fig. 4, the enhanced responses of HTE cells to asbestos appear to be a function of mineralogy and not fiber concentration. The same concentrations of fibers greater than 5 μm of chrysotile and crocidolite that cause proliferation in HTE cells result in no effects when comparable concentrations of FD14 fibers are used, insignificant cytotoxicity with S157 fibers, and significant cytotoxicity with CPS183 fibers. It there-

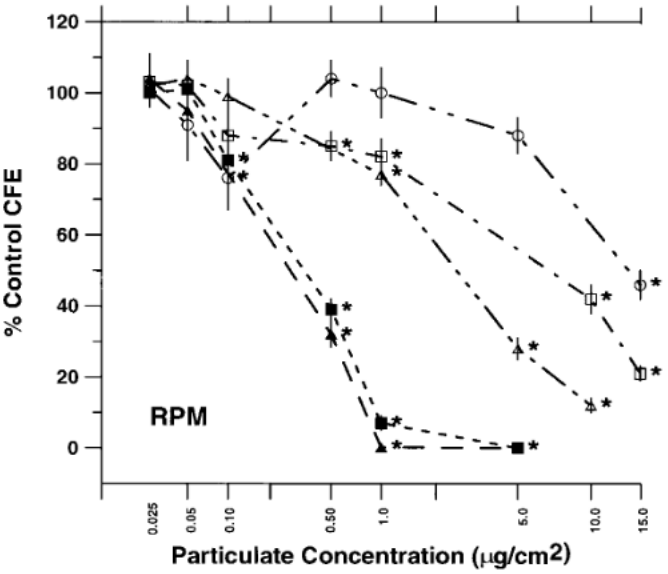

FIG. 3. Colony-forming efficiency (CFE) of RPM cells at various weight concentrations of samples. The standard error in CFE is indicated on the symbols. *$p <$ 0.05 in comparison to untreated controls. (▲) Chrysotile; (■) crocidolite; (○) FD14; (□) S157; (△) CPS183.

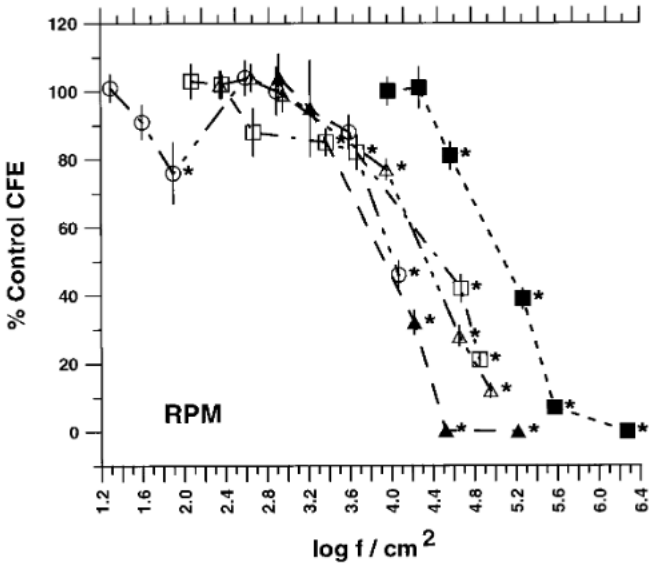

FIG. 5. Colony-forming efficiency (CFE) assays in RPM cells expressed as a function of fibers ≥5 μm in length and length:width ≥ 5:1 per cm^2 (f/cm^2). The symbol width is equal to or greater than estimated error in f/cm^2. The standard error in CFE is indicated on the symbols. *$p <$ 0.05 in comparison to untreated controls. (▲) Chrysotile; (■) crocidolite; (○) FD14; (□) S157; (△) CPS183.

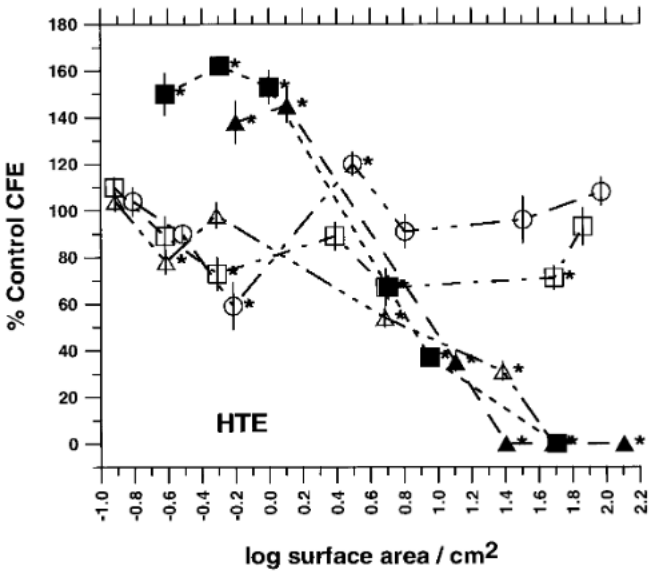

FIG. 6. Colony-forming efficiency (CFE) assays in HTE cells expressed as a function of surface areas of mineral samples (mm^2/cm^2). The symbol width is equal to or greater than one standard error in surface area/cm^2. The standard error in CFE is indicated on the symbols. *$p < 0.05$ in comparison to untreated controls. (▲) Chrysotile; (■) crocidolite; (○) FD14; (□) S157; (△) CPS183.

fore seems likely that characteristics of the samples that are related to their mineralogy contribute to proliferation and/or cell growth inhibition.

As shown in Fig. 5, the response of RPM cells appears to be independent of the mineralogy of the samples. Neglecting the slight cytotoxic response of FD14 at low concentrations, the minimum concentrations of fibers per square centimeter necessary to cause significant decreases in CFE is between 10^3 and 10^4 fibers per square centimeter for all samples. In changing the size definition of a fiber (e.g., >8, ≤0.25 μm; >20 μm, all widths; all lengths, w < 0.28 μm), we found that the effective dose changed but the relationships among the samples did not (data not shown).

Figures 6 and 7 show CFE data in HTE and RPM cells, respectively, as a function of surface area. It is evident that surface area per se cannot explain cellular responses to minerals in HTE or RPM cells. Despite the fact that crocidolite and chrysotile have much larger surface areas per microgram, the range in the amount of sample administered resulted in similar doses between the asbestos and talc samples.

DISCUSSION

Asbestos is a term applied to a group of minerals that possess similar physical properties because of their habit of growth. However, different types of asbestos differ in their mineralogy and fiber size, which in turn may vary in preparations obtained from different geographic locations and sometimes even from the same locality (Guthrie and Mossman, 1993). The two most widely studied types of asbestos are the serpentine mineral chrysotile ($Mg_3Si_2O_5(OH)_4$), the most common type of asbestos in the Northern hemisphere and in commercial usage historically, and the asbestiform variety of the amphibole riebeckite, crocidolite ($Na_2Fe_3^{2+}Fe_2^{3+}Si_8O_{22}(OH)_2$), a high-iron-containing asbestos mined in parts of South Africa and Western Australia. Although crocidolite is implicated as more potent in the induction of mesothelioma, both chrysotile and crocidolite are linked occupationally to the development of lung cancer and asbestosis (Mossman and Gee, 1989; Mossman *et al.,* 1990, 1996; Guthrie and Mossman, 1993; Health Effects Institute, 1991).

How asbestos causes lung disease is uncertain, but acute toxicity, measured by a variety of techniques which have detected increases in membrane permeability, necrosis, release of oxygen-free radicals, exfoliation, and cell death (reviewed in Mossman and Begin, 1989) has been observed in a variety of cells exposed to high concentrations of fibers. At lower concentrations, both crocidolite and chrysotile asbestos cause cell proliferation in HTE cells and organ cultures, phenomena not observed with various synthetic fibers or nonfibrous analogs of asbestos (Marsh and Mossman, 1988; Woodworth *et al.,* 1983). These biological responses to asbestos may be important in the induction of neoplasms as cell injury may cause exfoliation and compensatory hyperplasia of surrounding cell types which are more sensitive to genetic damage. As suggested by Ames and Gold (1990), mitogenesis may facilitate mutagenesis and contribute to tumor development. In addition, cell proliferation is an important component of tumor promotion and progression, and asbestos is a documented tumor promoter in epithelial cells of the respiratory tract (reviewed in Mossman *et al.,* 1990, 1996; Health Effects Institute, 1991).

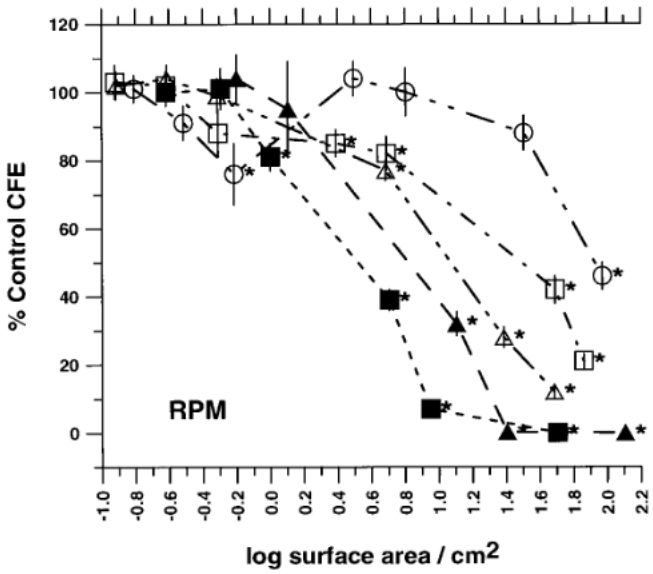

FIG. 7. Colony-forming efficiency (CFE) assays in RPM cells expressed as a function of surface areas of mineral samples (mm^2/cm^2). The symbol width is equal to or greater than one standard error in surface area/cm^2. The standard error in CFE is indicated on the symbols. *$p < 0.05$ in comparison to untreated controls. (▲) Chrysotile; (■) crocidolite; (○) FD14; (□) S157; (△) CPS183.

Our results with asbestos samples are interesting in that HTE cells are unique in exhibiting increased CFE, in comparison to untreated and talc-exposed cells. Moreover, both cell types were more sensitive to the cytotoxic effects of equal weight dose amounts of asbestos in comparison to talc. The lack of response of RPM cells to the proliferative effects of asbestos may reflect the fact that single cells, as opposed to confluent monolayers (Marsh and Mossman, 1988; Woodworth *et al.,* 1983), were exposed to fibers here. For example, when added to confluent, growth-arrested RPM cells, crocidolite causes cell proliferation as measured by dual fluorescence techniques with an antibody to 5-bromodeoxyuridine (BrdU) and the DNA dye YOYO (Goldberg *et al.,* 1997). Moreover, increased numbers of both pleural mesothelial and bronchial epithelial cells incorporating BrdU are observed after inhalation of NIEHS crocidolite or chrysotile by rats (BeruBe *et al.,* 1996). As suggested by Gerwin *et al.* (1987), mesothelial cells may require growth factors, either produced endogenously or produced by other cell types, for proliferative responses to asbestos, and the small numbers of cells used in the CFE bioassay may not be sufficient for amounts of cytokines needed here.

Our experiments also show that fibrous talc does not cause proliferation of HTE cells or cytotoxicity equivalent to asbestos in either cell type despite the fact that talc samples contain durable mineral fibers with dimensions similar to asbestos. These results are consistent with the findings of Stanton *et al.* (1981) who found no significant increases in pleural sarcomas in rats after implantation of materials containing fibrous talc. Moreover, Smith and colleagues report no sarcomas in hamsters after implantation of FD14 (1979), and other rodent studies in which talcs of various types have been administered by inhalation of injection also have not shown an increased incidence of mesotheliomas or carcinomas (Stenback and Rowland, 1978; Wehner *et al.,* 1977). Epidemiological studies also indicate that talc in a number of occupational settings is less pathogenic than asbestos in the development of lung cancer, and the reports indicating excess lung cancer mortality may underestimate smoking habits, an important confounder, and exposure to commercial asbestos (reviewed in IARC, 1987a,b; Ross *et al.,* 1993). In essence, data have not proven that talc is a human carcinogen as small numbers of cohorts have been studied, smoking histories are poorly documented, and workers were often exposed to other dusts, including asbestos, that may cause lung disease.

Increases in cytotoxicity over time with CPS183, as opposed to the other talc samples, in both cell types also suggest the importance of mineralogic differences as the size distributions of CPS183 and S157 are similar. Since CPS183 fibers are mainly talc, while S157 contains more talc/amphibole and amphibole, mineralogical variability may affect the responses of cells to cytotoxic effects of talc. Nonfibrous particles such as quartz may also play a role in cytotoxicity of the talc samples since CPS183 higher number of quartz particles, a mineral known to be cytolytic (Mossman and Begin, 1989).

Data presented here lend increased uncertainty to the concept that long thin fibers [length >8 μm, width ≤ 0.25 μm, i.e., the Stanton hypothesis (Stanton *et al.,* 1981)] are the predominant factors predicting tumorigenicity and fibrogenicity (Mossman *et al.,* 1990; Health Effects Institute, 1991). In his elegant and comprehensive studies, Stanton and colleagues implanted two samples of fibrous talc (No. 6 and No. 7 samples) into rats. One of us (AW) examined talc No. 6 and found it to be similar in mineralogy, size distribution, and morphology to FD14, and little is known about No. 7 except that it was obtained from the Gouverneur District. Neither talc produced significant excesses in pleural sarcomas despite the fact that the dose of fibers >8 μm in length and <0.25 μm in width in sample No. 6 was large enough to predict a tumor probability of $>50\%$.

In summary, intrapleural injection studies in rats, epidemiologic investigations, and our *in vitro* work with fibrous talc here suggest caution in generalizing that durable fibers >5 μm or with aspect ratios approximating Stanton criteria are always more bioreactive and pathogenic. Our work is significant in that it supports reanalysis of the Stanton data by Wylie *et al.* (1987) and others (Oehlert, 1991; Nolan and Langer, 1993) and provides data implicating the importance of mineral type, rather than fiber length per se, in determining cellular outcomes associated with pathogenicity of mineral dusts.

ACKNOWLEDGMENTS

Work was supported in part by a grant from NIEHS (R01ES06499) to BTM and from R. T. Vanderbilt Company to AGW and CS. We thank Dr. Cynthia Timblin for her input into interpretation of CFE data.

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